Exhibit 1

A Mechanism for the Specific Immunogenicity of Heat Shock Protein-Chaperoned Peptides

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ated cardiac damage in normal mice is not the result of viral-mediated cytolysis, but rather is from the actions of recruited inflammatory cells. The data are also consistent with, but do not prove, a requirement of MIP-1α in an autoimmune-mediated model of CVB3-induced myocarditis. In this regard, it is noteworthy that antibodies to MIP-1α reduce the severity of experimental autoimmune encephalomyelitis (8), and that the murine MIP-1α gene maps to a region of chromosome 11 that includes the Idd4 insulin-dependent diabetes locus (13).

We infected −/− mice with influenza virus to examine the inflammatory response to a pathogen of different tissue tropism. This model was chosen because pulmonary alveolar macrophages are a rich source of MIP-1α (14), suggesting that it may be involved in pulmonary inflammation. Influenza-infected mice were killed at day 6 or 7 p.i., when pathology is maximum (15). The lungs of most of the +/+ animals were inflamed and edematous; those of the −/− mice were less severely affected. Histologic sections of lung were graded for inflammation between 0+ and 4+ (16), on the basis of the extent of mononuclear cell infiltration and tissue damage (Fig. 3). The +/+ mice had significantly more inflammation than −/− mice ($P = 0.012$ by the Wilcoxon rank order test). These data demonstrate that MIP-1α contributes to influenza virus–induced pneumonitis.

Influenza virus is generally cleared from the lungs of immunocompetent mice by 6 to 10 days p.i. by a T cell–dependent mechanism (17). To determine whether MIP-1α affects influenza virus clearance, we measured viral titers at various times after infection and calculated the geometric mean for each genotype (Fig. 3D). Viral titers were higher in −/− animals compared with +/+ controls at day 3 p.i. ($P = 0.14$ by the two-tailed t test) and significantly so at days 6 and 7 p.i. ($P = 0.02$). Part of this difference may be accounted for by the increased weight of the +/+ lungs, but this weight difference was at most twofold, whereas the difference in viral titers was 100- to 1000-fold. By day 21 p.i., no virus was detected in either group of mice. This delay in T cell–dependent viral clearance in the −/− mice suggests that MIP-1α may be required for efficient recruitment of immunocompetent T cells to sites of viral infection, which is consistent with the ability of the chemokine to induce chemotaxis of T cells in vitro (2, 3).

Our experiments provide genetic evidence for the requirement of a β chemokine in inflammation and demonstrate that other chemokines do not functionally substitute for MIP-1α. The relative importance of MIP-1α in an inflammatory response may depend on the pathogen or tissue involved, possibly because of the expression of other molecules with compensatory activity. Nevertheless, the role of MIP-1α in diseases as different as myocarditis and pneumonitis suggests that this chemokine may also mediate inflammation in response to a variety of other stimuli.

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A Mechanism for the Specific Immunogenicity of Heat Shock Protein–Chaperoned Peptides

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Endogenously synthesized antigenic determinants are generally presented on major histocompatibility complex (MHC) class I molecules, whereas exogenous determinants are presented by MHC class II molecules. Here, it is shown that exogenous antigens chaperoned by a heat shock protein can be channeled into the endogenous pathway, presented by MHC class I molecules, and recognized by $CD8^+$ T lymphocytes. This pathway is functional only in a subset of macrophages among the cell types tested. These observations provide a basis for the tumor-specific and virus-specific immunogenicity of cognate heat shock protein preparations and offer a mechanism for the classical phenomenon of cross-priming.

Heat shock proteins (HSPs) isolated from cancer cells or virus-infected cells elicit protective immunity or cytotoxic T lymphocytes (CTLs) to the cognate tumor or viral antigen (1–3). In contrast, HSPs isolated from normal tissues do not elicit such immunity (2, 3). Because the HSP genes do not show tumor-associated DNA polymorphism, it has been suggested that HSPs derived from cancers or virus-infected cells are not immunogenic per se but rather chaperone tumor- or virus-specific antigenic peptides generated during antigen processing, and that it is the peptides and not the HSPs that are immunogenic (4). This suggestion was upheld by the sequencing of a number of HSP-associated peptides (5) and by the observation that HSPs stripped of associated peptides lose their immunogenicity (3).

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One of the unresolved questions in this area has been the mechanism whereby HSP-peptide complexes elicit specific immunity. Immunogenicity of HSP preparations is exquisitely dependent on the presence of functional phagocytic cells in the host; the depletion of such cells rendered the host incapable of being immunized by HSP preparations (6). This observation led to the suggestion that HSPs are taken up by the macrophage and are re-presented by the MHC class I molecules of the macrophage, which finally stimulate the appropriate T cells (7). Thus, a mechanism of indirect presentation of HSP-chaperoned peptides was invoked. The observations reported here support this mechanism.

We have investigated whether HSP-chaperoned peptides could be re-presented by phagocytic cells in a vesicular stomatitis virus (VSV) model. The HSP gp96 was isolated to apparent homogeneity (8) from EL4 cells transfected with the gene encoding the

nucleocapsid protein of VSV (N1 cells) (9). As a negative control, gp96 was also isolated from untransfected EL4 cells. Because soluble and particulate moieties are taken up by macrophages in essentially different ways, the gp96 preparations were centrifuged at 100,000g for 90 min to ensure that only soluble gp96 was used. The gp96 preparations were used to pulse macrophages in vitro that were obtained from the peritoneal exudate cells of C57BL/6 mice. We measured the ability of the pulsed macrophages to stimulate VSV-specific, K^b-restricted CTLs as a function of their ability to stimulate CTLs to release tumor necrosis factor (TNF) and their ability to act as targets of such CTLs. Macrophages pulsed with N1-derived gp96 could stimulate the release of TNF by VSV-specific CTLs, whereas those pulsed with EL4-derived gp96 could not (Fig. 1A). In cytotoxicity assays, macrophages pulsed with N1-derived gp96 could be lysed by VSV-specific CTLs, whereas those pulsed with EL4-derived gp96 were not (Fig. 1B). The quantity of N1-derived gp96 necessary to elicit a half-maximal response was found to be the same (1.5 to 3 μg/ml) for the two assays in any given experiment. Lysis of gp96-pulsed macrophages was inhibited by antibodies to K^b or to CD8, but not by antibodies to D^b or to CD4 (Fig. 1C).

The proportion of macrophages that were lysed never exceeded 30%, even at high effector-to-target (E/T) ratios (Fig. 2B). This persistently limited lysis suggests that only a subpopulation of macrophages can internalize and present HSP-chaperoned peptides in the context of MHC class I molecules. This idea is reminiscent of the observations of Rock and colleagues, who suggested that a small subpopulation of splenic macrophages can present exogenous antigens through the endogenous pathway (*10*). The possibility that only a specific macrophage population can present HSP-chaperoned peptides is supported by the observation that the macrophage line RAW309.Cr.1, which is functional in phagocytosis and pinocytosis and in antigen presentation to MHC class II–restricted T helper cells, could not present gp96-chaperoned peptides to VSV-specific CTLs (*11*). B cells and fibroblasts were also tested for their ability to present gp96-chaperoned peptides through the MHC class I molecules and were found to be unable to do so (*11*).

The above data indicate that N1-derived gp96 molecules are chaperoning the K^b epitope of VSV (or a precursor thereof) and that this epitope is being inducted into the endogenous presentation pathway of the macrophages. We tested this premise directly with the use of gp96 preparations reconstituted in vitro with the octamer K^b-binding epitope of VSV (VSV8). Macrophages pulsed with VSV8-gp96 complex, but not those pulsed with peptide A–gp96 complex, were recognized by VSV-specific CTLs (Fig. 2, A and B). Interestingly, a longer 20-mer peptide containing the K^b epitope, which cannot sensitize macrophages for CTL recognition by itself, did so quite effectively—and to an extent comparable to VSV8—when complexed with gp96 (Fig. 2C). To determine whether complexes of peptides with any protein might be able to sensitize macrophages for specific recognition, we tested peptides complexed with albumin or histone as in Fig. 2, A and B; no sensitizing effect was detected (*11*). A quantitative comparison of the ability of free and gp96-chaperoned VSV8 to stimulate CTLs showed that VSV8-gp96 complexes were 200 to 400 times more efficient at pulsing macrophages than was VSV8 alone; a free VSV8 concentration of ~50 nM was necessary to elicit the same level of CTL stimulation by pulsed macrophages as was elicited by 200 pM VSV8-gp96 complex (Fig. 2D)

We next examined the sensitivity of the macrophages' re-presentation of gp96-chaperoned peptides to a number of inhibitors (Table 1). Depletion of the intracellular adenosine triphosphate pools of the macro-

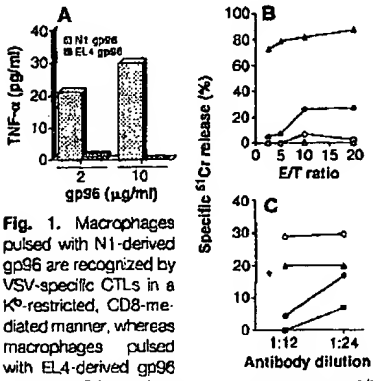

Fig. 1. Macrophages pulsed with N1-derived gp96 are recognized by VSV-specific CTLs in a K^b-restricted, CD8-mediated manner, whereas macrophages pulsed with EL4-derived gp96 are not. (**A**) Pristane-induced macrophages (1×10^4) from C57BL/6 mice and VSV-specific CTLs (5×10^4) were cocultured in the presence of gp96 (2 or 10 μg/ml) derived from N1 or EL4 cells in 96-well U-bottom plates at 37°C. After 24 hours, supernatants were collected and TNF-α production was measured by bioassay in a cytotoxicity assay with WEHI164 cells (*17*). (**B**) The ability of gp96-pulsed macrophages to act as targets in CTL assays was tested. Macrophages (5×10^6 per milliliter) were pulsed with gp96 (10 μg/ml) derived from N1 cells (●) or EL4 cells (○), with VSV nucleocapsid K^b epitope peptide (10 μM) (▲) as a positive control, or with a medium control (△) for 2 hours at 37°C, followed by labeling with ^{51}Cr for 1.5 hours. These cells were used as targets in a 4-hour ^{51}Cr release assay with VSV-specific CTLs. (**C**) CD4 monoclonal antibody (mAb GK1.5) ascites (○) (obtained from E. Nakayama, Okayama University School of Medicine, Okayama, Japan), CD8 mAb (YTS169.4) (■), H-2K^b mAb (Y3) (●), H-2D^b mAb (B22.249) (△), or RPMI 1640 control (×) were added to the CTL assay at the same time as effector cells and ^{51}Cr-labeled macrophages pulsed with N1 gp96 (E/T ratio = 10). See (*18*) for additional specificity analysis

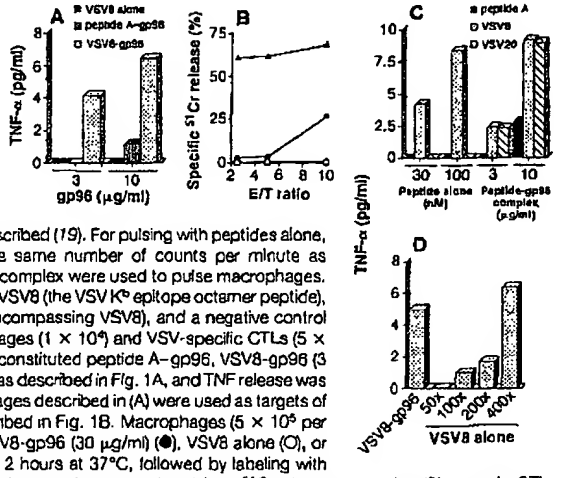

Fig. 2. Macrophages pulsed with in vitro reconstituted complexes of gp96 with the K^b epitope of VSV are recognized by VSV-specific CTLs. Macrophages were pulsed with free peptides or in vitro reconstituted complexes of gp96 with experimental or control peptides, as described (*19*). For pulsing with peptides alone, free peptides containing the same number of counts per minute as present in the gp96-peptide complex were used to pulse macrophages. The peptides used (*20*) were VSV8 (the VSV K^b epitope octamer peptide), VSV20 (a 20-mer peptide encompassing VSV8), and a negative control VSV peptide A. (**A**) Macrophages (1×10^4) and VSV-specific CTLs (5×10^4) were cocultured with reconstituted peptide A–gp96, VSV8-gp96 (3 or 10 μg/ml), or VSV8 alone, as described in Fig. 1A, and TNF release was monitored. (**B**) The macrophages described in (A) were used as targets of VSV-specific CTLs, as described in Fig. 1B. Macrophages (5×10^5 per 100 μl) were pulsed with VSV8-gp96 (30 μg/ml) (●), VSV8 alone (○), or liver gp96 (30 μg/ml) (■) for 2 hours at 37°C, followed by labeling with ^{51}Cr for 1.5 hours. They were then used as targets in a 4-hour ^{51}Cr release assay with VSV-specific CTLs. As a positive control, N1 cells were used as targets (▲). (**C**) Free and gp96-complexed VSV8 and VSV20 and gp96-complexed peptide A (negative control) were used to pulse macrophages at 3 and 10 μg/ml, and their ability to stimulate VSV-specific CTLs was monitored. (**D**) The ability of VSV8-gp96 complexes to pulse macrophages so as to enable them to stimulate CTLs was compared quantitatively to the corresponding ability of free VSV8 alone. Macrophages were pulsed with VSV8-gp96 complex (3 μg of gp96 per milliliter, or 31 nM) or the indicated relative quantities of free VSV8 (1× free VSV8 concentration refers to the concentration of VSV8 present in the VSV8-gp96 complex at 3 μg/ml and is equivalent to ~200 pM). TNF production by sensitized macrophages was measured as described in Fig. 1.

phages by pretreatment with sodium azide and 2-deoxyglucose was inhibitory, whereas chloroquine, which inhibits processing in an acidic microenvironment, had no effect. In contrast, pretreatment of macrophages with brefeldin A (BFA) abrogated re-presentation. Apparently, gp96-peptide complexes are internalized by macrophages (that is, processed through nonacidic compartments) and the gp96-peptide complexes or the peptides alone are routed through the endoplasmic reticulum (ER) by a mechanism dependent on (*10*) or independent (*12*) of transport-associated proteins.

Several lines of evidence indicate that gp96-chaperoned peptides are processed internally and are re-presented by the MHC class I molecules of the macrophages, and that the effects reported here are not a consequence of surface phenomena: (i) Sensitization of surface MHC class I molecules is limited by the size of peptide, whereas the gp96-chaperoned peptides show no such preference. (ii) The gp96-chaperoned peptides are several hundred times as effective as free peptides in sensitizing macrophages for CTL recognition. (iii) Although free peptides sensitize all cell types for CTL recognition, sensitization by gp96-chaperoned peptides is limited to a subset of macrophages. (iv) The processing of gp96-peptide complexes in the macrophages is sensitive to BFA. Collectively, these observations begin to shed light on the long-standing puzzle of how immunization of mice with HSP-peptide complexes elicits tumor-specific and virus-specific CTL response (*1–3*).

Together with the observations that HSPs bind antigenic peptides and that the ER-resident HSP gp96 associates with MHC class I molecules (*13*), the lack of allelic variation in HSP genes (*14*) had previously led us to predict that HSP-associated peptides would serve as precursors of peptides associated with any given MHC class I and that HSPs will be able to cross-prime (*7*). This prediction has been tested by two independent approaches. In the first approach, gp96 isolated from VSV-infected cells of $H\text{-}2^b$ haplotype (EL4 cells) and $H\text{-}2^d$ haplotype (Meth A cells) was used to sensitize macrophages (of $H\text{-}2^b$ haplotype) for recognition by VSV-specific CTLs of $H\text{-}2^b$ haplotype. The gp96 preparations isolated from VSV-infected cells of either haplotype were equally efficient at sensitization (Fig. 3A). In the second approach, gp96 preparations from VSV-infected cells of the $H\text{-}2^b$ or $H\text{-}2^d$ haplotypes were used to immunize $H\text{-}2^b$ haplotype mice and the mice were tested for $H\text{-}2^b$–restricted CTL response. The gp96 preparations from VSV-infected cells of either haplotype elicited a specific CTL response (Fig. 3B). These experiments indicate that peptides chaperoned by gp96 are indeed unselected with respect to the MHC haplotype. Further, as predicted (*7*), these experiments demonstrate cross-priming by HSP preparations and suggest that HSPs can act as mediators of this classical phenomenon (*15*).

Table 1. The re-presentation of gp96-chaperoned peptides by macrophages is inhibited by BFA and by sodium azide plus 2-deoxyglucose, but not by chloroquine. Inhibitors were used as described (*16*).

Pretreatment	Pulsing with VSV8		Pulsing with N1 gp96	
	Cytotoxicity (%)	Inhibition (%)	Cytotoxicity (%)	Inhibition (%)
	Experiment 1			
None	90.0		26.0	
BFA	85.0	5.6	6.0	76.9
	Experiment 2			
None	94.0		26.2	
Azide + 2-deoxyglucose	74.5	20.7	0	100
Chloroquine	75.0	20.2	22.3	14.2

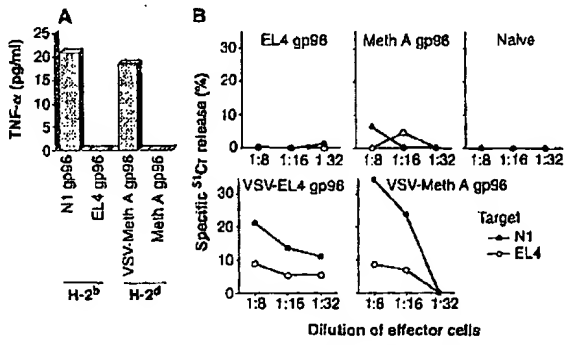

Fig. 3. (**A**) The gp96 preparations isolated from VSV-infected cells of the $H\text{-}2^b$ or $H\text{-}2^d$ haplotypes can be processed by macrophages of the $H\text{-}2^b$ haplotype to stimulate K^b-restricted VSV-specific CTLs. Macrophages ($H\text{-}2^b$) were pulsed with gp96 (3 μg/ml) derived from uninfected or VSV-infected Meth A ($H\text{-}2^d$) cells or from EL4 ($H\text{-}2^b$) or N1 ($H\text{-}2^b$) cells and were used to stimulate K^b-restricted CTLs as described in Fig. 1A. (**B**) Preparations of gp96 isolated from VSV-infected cells of the $H\text{-}2^b$ or $H\text{-}2^d$ haplotypes can prime mice of the $H\text{-}2^b$ haplotype for generation of VSV-specific CTLs. C57BL/6 mice ($H\text{-}2^b$) were vaccinated with gp96 derived from uninfected or VSV-infected Meth A ($H\text{-}2^d$) or EL4 ($H\text{-}2^b$) cells. Spleens of immunized mice were tested for cytotoxicity against N1 cells or EL4 cells (*21*).

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16. Macrophages (5 × 10^6 per milliliter) were preincubated with sodium azide (30 mM) plus 2-deoxyglucose (5 mM) for 1.5 hours, followed by pulsing with N1 gp96 (10 μg) or VSV8 (10 μM) for 2 hours at 37°C. The pulsed cells were labeled with ^{51}Cr for 1.5 hours and used as target cells in a 4-hour ^{51}Cr release assay with VSV-specific CTLs in the absence of inhibitors. For inhibition with chloroquine (100 μM), cells were similarly treated except that the CTL assay was carried out for only 2 hours; this duration was predetermined to be sufficient to detect significant CTL response. Also, the effect of chloroquine was not fully reversible in this duration, as determined by the use of an MHC class II–restricted line against an unrelated antigen. In experiments with BFA (1.5 μg/ml), the inhibitor was present throughout the experiment, although its concentration was reduced by half during the CTL assay. This concentration of BFA was predetermined to have no direct inhibitory effect on CTLs. E/T ratios were 12 for experiment 1 and 20 for experiment 2.
17. WEHI164 cells were seeded (2500 cells per well) in flat-bottom 96-well plates. Serially diluted supernatants of macrophage-CTL coculture were added; recombinant TNF-α was added to WEHI164 in separate wells as control. After 4 hours of culture at 37°C, 50 μl of 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT, 1 mg/ml) was added, with 4 hours of incubation, followed by 100 μl of propanol and 0.05 M HCl. Optical density (590 nm) was measured immediately. Sample concentrations were calculated by comparison with dilution points, which resulted in killing of 50% of WEHI164 cells.
18. We also tested the specificity of the ability of gp96-

pulsed macrophages by using the macrophages pulsed with either gp96 preparation to stimulate CTLs against an irrelevant tumor. None of the pulsed macrophages could stimulate the tumor-specific CTLs.

19. For in vitro reconstitution of gp96-peptide complexes, gp96 derived from normal liver (50 μg) and ^{125}I-labeled peptides (5 μg) were incubated at 50°C for 10 min followed by room temperature for 30 min. Free peptides were removed by extensive washing with Microcon 50 (Amicon), such that no free peptides were detected on SDS–polyacrylamide gel electrophoresis of the complexes (Z. Li, R. Suto, P. K. Srivastava, in preparation).
20. The sequence of VSV20 is Ser-Leu-Ser-Asp-Leu-Arg-Gly-Tyr-Val-Tyr-Gln-Gly-Leu-Lys-Ser-Gly-Asn-Val-Ser-Cys. The sequence of the negative control VSV peptide A is Lys-Arg-Gln-Ile-Tyr-Thr-Asp-Leu-Glu-Met-Asn-Arg-Leu-Gly-Lys.
21. C57BL/6 mice (*H-2*^{b} haplotype) were subcutaneously injected twice at a 7-day interval with gp96 (10 μg in phosphate-buffered saline) derived from uninfected or VSV-infected Meth A cells or EL4 cells. Seven days after the second vaccination, spleens were removed and spleen cells (8 × 10^{6} cells per well) were cocultured in mixed lymphocyte-tumor culture (MLTC) with irradiated N1 cells (1.4 × 10^{5} cells per well) in 24-well plates. On day 7, each well was harvested. Serially diluted culture cells were tested against N1 cells or EL4 cells for cytotoxicity in a ^{51}Cr release assay.
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Human H-Y: A Male-Specific Histocompatibility Antigen Derived from the SMCY Protein

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H-Y is a transplantation antigen that can lead to rejection of male organ and bone marrow grafts by female recipients, even if the donor and recipient match at the major histocompatibility locus of humans, the HLA (human leukocyte antigen) locus. However, the origin and function of H-Y antigens has eluded researchers for 40 years. One human H-Y antigen presented by HLA-B7 was identified as an 11-residue peptide derived from SMCY, an evolutionarily conserved protein encoded on the Y chromosome. The protein from the homologous gene on the X chromosome, *SMCX*, differs by two amino acid residues in the same region. The identification of H-Y may aid in transplantation prognosis, prenatal diagnosis, and fertilization strategies.

Histocompatibility antigens that can induce transplant rejection include the class I and class II molecules of the major histocompatibility complex (MHC), as well as a large number of so-called minor histocompatibility (H) antigens. In mice, the use of inbred strains has shown that minor H antigens are encoded by almost 50 different allelically polymorphic loci scattered throughout the genome (*1*). Humans also have minor H antigens although their overall number and complexity remains uncertain. Both species have the male specific antigen H-Y (*2*, *3*). H-Y was initially identified through the observation that within an inbred mouse strain, most of the male-to-female skin grafts were rejected, whereas transplants in other sex combinations nearly always succeeded (*2*). In humans, sex mismatch is a significant risk factor associated with rejection or the development of graft-versus-host disease in bone marrow transplant recipients (*3*–*6*). The H-Y antigen is expressed in most different human tissues (*4*, *7*), and H-Y specific immune responses occur during the transplantation of other organs, blood transfusion, and pregnancy (*8*).

As with other minor H antigens, the recognition of H-Y by T lymphocytes is MHC-restricted (*3*, *9*), and some H-Y antigens are peptides derived from cellular proteins that are presented on the cell surface in association with MHC class I molecules (*10*). We have developed a technique for the identification of individual peptides that are bound to MHC molecules and recognized as antigens by T cells. By combining microcapillary liquid chromatography–electrospray ionization mass spectrometry with T cell epitope reconstitution assays (*11*–*13*) we now report the identification of a peptide antigen recognized by a human cytotoxic T lymphocyte (CTL) clone that is H-Y–specific and restricted by the class I MHC molecule HLA-B7.

To isolate endogenously processed H-Y peptides, HLA-B7 molecules were purified by affinity chromatography from the H-Y positive, B lymphoblastoid cell line JY (*14*). The associated peptides were extracted in acid and separated from high molecular weight material by ultrafiltration (*15*) and subsequently fractionated by reverse-phase high-pressure liquid chromatography (HPLC) (*11*). Samples of each fraction were incubated with HLA-B7^{+}, H-Y^{-} T2-B7 target cells to assay for reconstitution of the epitope recognized by

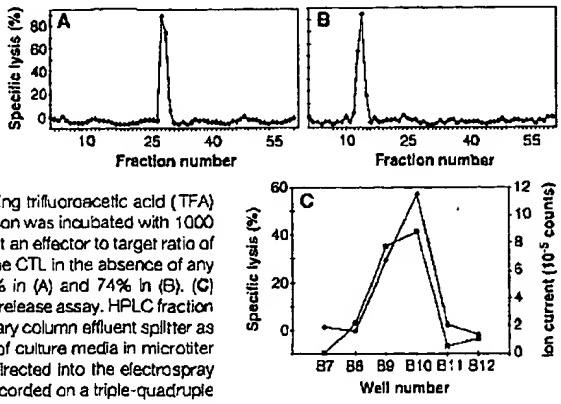

Fig. 1. Reconstitution of the H-Y epitope with HPLC-fractionated peptides extracted from HLA-B7 molecules. (**A**) HLA-B7 molecules were immunoaffinity purified form 2 × 10^{10} H-Y^{+} JY cells. Peptides were eluted from B7 molecules with 10% acetic acid, pH 2.1, filtered through a 5-kD cut-off filter and fractionated on a C18 reverse phase column. Buffer A was 0.1% heptafluorobutyric acid (HFBA) and buffer B was 0.1% HFBA in acetonitrile. The gradient consisted of 100% buffer A (0 to 20 min), 0 to 12% buffer B (20 to 25 min), and 12 to 50% buffer B (25 to 80 min) at a flow rate of 200 μl/min. Sixty fractions of 200 μl each were collected from 20 to 80 min. (**B**) Fractions 28 and 29 from the separation shown in (A) were rechromatographed with the same acetonitrile gradient, but using trifluoroacetic acid (TFA) instead of HFBA as the organic modifier. For both panels, 3% of each peptide fraction was incubated with 1000 ^{51}Cr-labeled T2-B7 cells at room temperature for 2 hours. CTLs were then added at an effector to target ratio of 10 to 1 and further incubated at 37°C for 4 hours. Background lysis of T2-B7 by the CTL in the absence of any peptides was −3% in (A) and −4% in (B); positive control lysis of JY was 75% in (A) and 74% in (B). (**C**) Determination of candidate H-Y peptide by mass spectrometry combined with ^{51}Cr release assay. HPLC fraction 14 from the separation in Fig. 1B was chromatographed with an on-line microcapillary column effluent splitter as previously described (*11*, *13*). One-fifth of the effluent was deposited into 100 μl of culture media in microtiter plate wells for analysis with CTLs. The remaining four-fifths of the material were directed into the electrospray ionization source, and mass spectra of the peptides deposited in each well were recorded on a triple-quadruple mass spectrometer (Finnigan-MAT, San Jose, California). (◆), H-Y epitope reconstitution activity measured as percent specific lysis; (■), abundance of peptide 1171 measured as ion current at m/z 391.